

Cigarette Smoking Is Associated With a Dose-Response Effect in Clopidogrel-Treated Patients With Diabetes Mellitus and Coronary Artery Disease

Results of a Pharmacodynamic Study

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Objectives This study sought to assess the presence of a dose-response effect of cigarette smoking and its impact on high on-treatment platelet reactivity (HPR) in patients with diabetes mellitus treated with clopidogrel.

Background Cigarette smoking is an inducer of cytochrome P450 1A2, a hepatic enzyme involved in clopidogrel metabolism. If cigarette smoking is associated with a dose-response effect on pharmacodynamic measures in clopidogrel-treated patients is unknown.

Methods A total of 134 type 2 diabetes mellitus patients on maintenance aspirin and clopidogrel therapy were studied. Patients were divided into 3 groups according to cotinine levels: <3 ng/ml (nonsmokers), 3 to 199 ng/ml (light smokers), and ≥200 ng/ml (heavy smokers). Platelet function was assessed by light transmittance aggregometry, VerifyNow P2Y₁₂ assay (Accumetrics, San Diego, California), and vasodilator-stimulated phosphoprotein. Rates of HPR were defined using established cutoff values.

Results A dose-response effect was observed for all pharmacodynamic parameters tested. Serum cotinine levels were inversely associated with platelet reactivity as assessed by light transmittance aggregometry using 5 and 20 μmol/l adenosine diphosphate ($p < 0.0001$ for all). Accordingly, platelet disaggregation increased with levels of serum cotinine ($p < 0.0001$). Similar results were found with P2Y₁₂ reaction units ($p < 0.0001$) and inhibition of platelet aggregation ($p = 0.005$) as defined by VerifyNow P2Y₁₂ testing, and platelet reactivity index ($p = 0.002$) as assessed by vasodilator-stimulated phosphoprotein. Higher serum cotinine levels were significantly associated with lower rates of HPR, as defined according to various pharmacodynamic cutoff measures.

Conclusions Cigarette smoking is associated with a dose-response effect on clopidogrel-induced antiplatelet effects and lower rates of HPR in diabetes mellitus patients. (J Am Coll Cardiol Intv 2012;5:293–300) © 2012 by the American College of Cardiology Foundation

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Numerous investigations have shown a broad variability in clopidogrel-induced antiplatelet effects, and patients with high on-treatment platelet reactivity (HPR) have an increased risk of recurrent atherothrombotic events (1,2). Multiple factors have been associated with the degree of platelet inhibition induced by clopidogrel. Among these, genetic and environmental factors modulating hepatic metabolism of clopidogrel appear to have a pivotal role (1,2). Clopidogrel is a prodrug that requires a 2-step oxidation by cytochrome P450 (CYP) isoenzymes to generate an active metabolite that in turn irreversibly inhibits the platelet

Abbreviations and Acronyms

ADP = adenosine diphosphate

CI = confidence interval

CYP = cytochrome P450

DM = diabetes mellitus

HPR = high on-treatment platelet reactivity

IPA = inhibition of platelet aggregation

LPA = late values of on-treatment platelet aggregation

LTA = light transmittance aggregometry

MFI = mean fluorescence intensity

MPA = maximal values of on-treatment platelet aggregation

OR = odds ratio

PD = pharmacodynamic

PGE₁ = prostaglandin E₁

PRI = platelet reactivity index

PRP = platelet-rich plasma

PRU = P2Y₁₂ reaction units

VASP-P = phosphorylation of vasodilator-stimulated phosphoprotein

P2Y₁₂ receptor (3). Cigarette smoking is a known inducer of CYP1A2, which is the predominant isoenzyme responsible for the first oxidative step in the conversion of clopidogrel into its active metabolite (4,5). Pharmacodynamic (PD) and clinical studies have shown that smokers treated with clopidogrel have enhanced platelet inhibition and derive higher relative benefit, as assessed by angiographic and clinical outcomes, than nonsmokers do (6–9). However, these studies identified the aforementioned effects in smokers consuming above a certain threshold of number of cigarettes and were not able to determine a dose-response effect in a continuous way. This may be attributed to the fact that these investigations were based on self-reported smoking, which is not an objective measure of the amount of nicotine exposure, as it depends for instance on the type and brand of cigarettes and smokers' habit (e.g., deep inhalation). In addition, because baseline characteristics are associated with variations in clopidogrel metabolism, it cannot be excluded that patient selection may have had an impact on these findings.

In the present investigation, the impact of cigarette smoking on clopidogrel-induced antiplatelet effects was assessed by means of a more objective assessment based on levels of serum cotinine, the major stable degradation product of nicotine metabolism (10). Because clopidogrel metabolism is reduced among patients with diabetes mellitus (DM), which may contribute to their high prevalence of HPR while on clopidogrel therapy (11), this population was identified to test our study hypothesis. The aim of the

present investigation was to assess if there is a dose-response effect of cigarette smoking, as assessed by serum cotinine levels, and how this affects rates of HPR in patients with DM on maintenance clopidogrel therapy.

Methods

Patient population. The present investigation is a cross-sectional observational study that evaluated the association between cigarette smoking and PD effects of clopidogrel. A database of patients who had undergone platelet function assessments at our Thrombosis Research Laboratory (University of Florida College of Medicine–Jacksonville) between 2006 and 2010 was used to identify eligible subjects for this investigation. Patients meeting study inclusion criteria, who also had a serum sample collected at the time of platelet function assessment to enable cotinine measurement, were identified. All patients had undergone percutaneous coronary intervention with stent implantation and were treated with dual antiplatelet therapy per standard of care. In particular, patients were eligible for the study if they had type 2 DM and were clinically stable while on maintenance dual antiplatelet therapy with aspirin (81 mg daily) and clopidogrel (75 mg daily) for at least 1 month. Patients needed to be on maintenance dual antiplatelet therapy for at least 1 month as prior investigations have shown that platelet reactivity is subject to variability in the earlier phases of treatment and reaches a steady-state phase following 1 month of therapy (12–14). Type 2 DM patients also needed to have been medically managed (oral or insulin therapy) for at least 2 months without changes in hypoglycemic treatment regimen. General major exclusion criteria included: known allergies to aspirin or clopidogrel; left ventricular ejection fraction <30%; blood dyscrasia; active bleeding or bleeding diathesis; gastrointestinal bleed within last 6 months; hemodynamic instability; cerebrovascular accident within 3 months; any malignancy; concomitant use of other antithrombotic drugs (oral anticoagulants, dipyridamole, ticlopidine, or cilostazol); recent treatment (<30 days) with a glycoprotein IIb/IIIa antagonist; platelet count <100 × 10³/μl; liver disease (baseline alanine transaminase >2.5× the upper limit of normal).

Patients were recruited at the Division of Cardiology of the University of Florida College of Medicine–Jacksonville. The study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Florida College of Medicine–Jacksonville. All subjects provided written informed consent for platelet function assessments and for storage of serum samples. The authors had full access to the data and take full responsibility for its integrity. All authors have read and agreed to the manuscript as written.

Blood sampling and functional assessments. Peripheral venous blood samples were drawn with a loose tourniquet to

avoid artifacts through a short venous catheter inserted into a forearm vein. Samples were collected before administration of the morning dose of clopidogrel (trough levels). The first 2 to 4 ml of blood was discarded to avoid spontaneous platelet activation. Samples were processed within 1 h after blood drawing.

Light transmittance aggregometry. Platelet aggregation was performed using light transmittance aggregometry (LTA) according to standard protocols (15–17). In brief, platelet aggregation was assessed using platelet-rich plasma (PRP) by the turbidimetric method in a 2-channel aggregometer (Chrono-Log 490 Model, Chrono-Log Corp., Havertown, Pennsylvania). PRP was obtained as a supernatant after centrifugation of citrated blood at 800 revolutions/min for 10 min. The isolated PRP was kept at 37°C before use. Platelet-poor plasma was obtained by a second centrifugation of the blood fraction at 2,500 revolutions/min for 10 min. Light transmission was adjusted to 0% with PRP and to 100% for platelet-poor plasma for each measurement and assessed following challenge with adenosine diphosphate (ADP) (5 and 20 $\mu\text{mol/l}$) (15–17). Maximal (MPA) and late (LPA) values of on-treatment platelet aggregation were measured. Percentage of platelet disaggregation was derived from MPA and LPA values [disaggregation (%) = $100 \times (1 - \text{LPA}/\text{MPA})$], as previously defined (15,16).

VerifyNow P2Y₁₂ assay. The VerifyNow P2Y₁₂ assay (Accumetrics, San Diego, California) is a rapid whole-blood point-of-care assay and was used according to the instructions of the manufacturer (16,17). The VerifyNow P2Y₁₂ assay reports the results as P2Y₁₂ reaction units (PRU). This assay mimics turbidimetric aggregation and uses disposable cartridges containing 20 mmol/l ADP and 22 nmol/l prostaglandin E₁ (PGE₁). Aggregation testing using ADP as a sole agonist activates P2Y₁ and P2Y₁₂ purinergic signaling, whereas adding PGE₁ increases the specificity of the test for P2Y₁₂ signaling. In a separate channel of the cartridge in which iso-TRAP is used as an agonist, a baseline value for platelet function is obtained, enabling assessment of inhibition of platelet aggregation (IPA) without having to wean the patient off antiplatelet treatment.

P2Y₁₂ reactivity index. The platelet reactivity index (PRI) was calculated as a measure of the functional status of the P2Y₁₂ signaling pathway. PRI was determined through assessment of phosphorylation status of vasodilator-stimulated phosphoprotein (VASP-P), a key, specific intraplatelet mediator of P2Y₁₂ signaling, according to standard protocols (15–17). In brief, VASP-P was measured by quantitative flow cytometry (Beckman Coulter FC500, Miami, Florida) using commercially available labeled monoclonal antibodies (Biocytex Inc., Marseille, France). The PRI was calculated after measuring the mean fluorescence intensity (MFI) of VASP-P levels following challenge with PGE₁ and PGE₁ plus ADP. PGE₁ increases VASP-P levels through stimulation of adenylate cyclase, whereas ADP

binding to purinergic receptors leads to inhibition of adenylate cyclase. Therefore, the addition of ADP to PGE₁-stimulated platelets reduces levels of PGE₁-induced VASP-P. The PRI was calculated as follows: $([\text{MFI PGE}_1] - [\text{MFI PGE}_1 + \text{ADP}]) / [\text{MFI PGE}_1] \times 100$. Elevated PRI values indicate up-regulation of the P2Y₁₂ signaling pathway (15–17).

Cotinine measurement. Cotinine levels were measured as a final batch assessment using stored serum samples collected at the time of platelet function assessment using the Cotinine Blood Test kit (Calbiotech, Spring Valley, California), a solid phase competitive enzyme-linked immunosorbent assay, as previously described (18). The samples and cotinine enzyme conjugate are added to the wells coated with anticotinine antibody. Cotinine in the samples competes with a cotinine enzyme conjugate for binding sites. Unbound cotinine and cotinine enzyme conjugate is washed off by a washing step. With the addition of the substrate, the intensity of color is inversely proportional to the concentration of cotinine in the samples obtained with the cotinine blood test. A standard curve is prepared relating color intensity to the concentration of the cotinine (18).

Definitions. Patients were divided into 3 groups according to serum cotinine levels measured by the cotinine enzyme-linked immunosorbent assay test. Serum cotinine levels <3, 3 to 199, and ≥ 200 ng/ml indicated nonsmoker, light smoker, and heavy smoker status, respectively (19–21).

HPR was defined using various previously defined cutoff levels that have been associated with an increased risk of recurrent ischemic events (1,15,22,23). These included the following cutoff values using LTA: MPA-ADP (20 $\mu\text{mol/l}$) >50% and MPA-ADP (5 $\mu\text{mol/l}$) >46%; VerifyNow P2Y₁₂ assay: PRU >230 and IPA <40%; and VASP: PRI >50%.

Statistical analysis. Continuous variables were analyzed for a normal distribution with the Kolmogorov-Smirnov test and presented as mean \pm SD or as median and interquartile range if a normal distribution was present or not, respectively. Student t test or Mann-Whitney U test were used for comparisons of continuous variables where appropriate. Categorical variables are expressed as frequencies and percentages. Categorical variables were tested using the chi-square test or Fisher exact test when at least 25% of values showed an expected cell frequency below 5. Analysis of variance with post hoc Bonferroni correction was used to compare continuous variables among more than 2 groups and correct for multiple comparisons. In addition, p values for trend when assessing platelet reactivity according to the smoking degree, which was considered as a categorical variable with an ordinal scale, were performed with a polynomial contrast with analysis of variance method, using median values of each category as coefficients. Comparisons between categorical variables were performed using McNemar test or binomial exact test. Control for potential confounders and analysis of independent correlates of HPR were performed with a logistic regression model, including

	Nonsmoker (n = 85)	Light Smoker (n = 27)	Heavy Smoker (n = 22)	p Value
Age, yrs	62.3 ± 9.0	64.0 ± 9.1	57.6 ± 9.1	0.04
Male	41 (48)	16 (59)	13 (59)	0.47
Race				0.67
Caucasian	52 (61)	20 (74)	16 (73)	
African American	29 (34)	6 (22)	5 (23)	
Hispanic	2 (2)	0	1 (5)	
Asian	2 (2)	1 (4)	0 (0)	
Risk factors/medical history				
Hyperlipidemia	79 (95)	25 (93)	20 (91)	0.72
Hypertension	82 (97)	27 (100)	22 (100)	0.41
Creatinine >1.5 mg/dl	11 (13)	3 (11)	0 (0)	0.35
Body mass index, kg/m ²	34.7 ± 7.7	31.7 ± 6.7	32.1 ± 7.7	0.13
Hemoglobin A _{1c}	7.7 ± 2.3	7.4 ± 1.5	7.6 ± 1.7	0.89
Prior myocardial infarction	50 (59)	16 (59)	18 (82)	0.13
Prior CABG	26 (31)	10 (37)	3 (14)	0.18
Prior stroke	5 (6)	2 (7)	2 (9)	0.87
Treatment				
Beta-blockers	71 (84)	21 (78)	15 (68)	0.27
Nitrates	27 (32)	10 (37)	7 (32)	0.87
ACE inhibitors/ARB	75 (88)	20 (74)	17 (77)	0.15
PPI	21 (25)	7 (26)	5 (23)	0.97
Statin	79 (93)	23 (85)	17 (77)	0.09
Insulin therapy	35 (41)	7 (26)	8 (36)	0.36

Values are mean ± SD or n (%).

ACE = angiotensin-converting enzyme; ARB = angiotensin II receptor blockers; CABG = coronary artery bypass graft; PPI = proton pump inhibitors.

age, insulin treatment, body mass index, creatinine >1.5 mg/dl, hemoglobin A_{1c}, use of statin, and proton pump inhibitors as covariates, and the degree of smoker (non-, light, or heavy smoker) as the independent categorical variable of interest, using nonsmoker as the reference category. Odds ratio (OR) and 95% confidence interval (CI) were calculated. All univariate variables $p < 0.1$ and those deemed of clinical interest were included in the statistical model. All probability values reported are 2-sided, and a value of $p < 0.05$ was considered significant. Statistical analysis was performed with SPSS software (version 15.0, SPSS Inc., Chicago, Illinois).

Results

A total of 134 type 2 DM patients with stable coronary artery disease on aspirin and clopidogrel therapy meeting study inclusion criteria were identified for this investigation. A total of 49 patients (37%) were active smokers. Patients were divided into 3 groups according to serum cotinine levels: <3 ng/ml (nonsmoker; n = 85), 3 to 199 ng/ml (light smoker; n = 27), and ≥200 ng/ml (heavy smoker; n = 22). Baseline demographics and clinical characteristics of the study population are provided in Table 1. Patients

were similar for all baseline characteristics, except for a lower age in the heavy smoker group ($p = 0.04$).

A dose-response effect was observed for all pharmacodynamic parameters tested. Serum cotinine levels were inversely associated with levels of on-treatment platelet reactivity as assessed by LTA for both MPA and LPA values using 5 and 20 $\mu\text{mol/l}$ ADP (p for trend <0.0001) (Fig. 1). Accordingly, platelet disaggregation increased with levels of serum cotinine (p for trend <0.0001; both 5 and 20 $\mu\text{mol/l}$ ADP; data not shown). Similarly to the LTA findings, results obtained with the VerifyNow P2Y12 assay also showed a dose-response effect as measured by PRU (p for trend <0.0001) and IPA (p for trend = 0.002) values (Fig. 2). Ultimately, enhanced clopidogrel-induced antiplatelet effects with increased cotinine levels were observed using flow cytometric assessment of VASP to define PRI values (p for trend = 0.001) (Fig. 3).

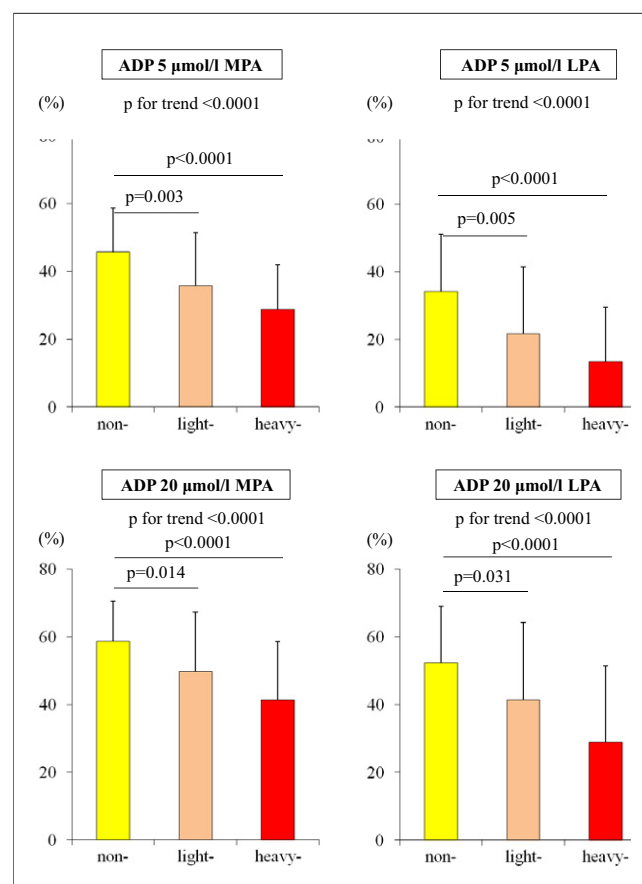


Figure 1. Platelet Reactivity Assessed by LTA According to the Degree of Smoking Defined by Cotinine Levels

Impact of the degree of smoking status to clopidogrel-induced antiplatelet effects on maximum and late 5 $\mu\text{mol/l}$ and 20 $\mu\text{mol/l}$ adenosine diphosphate (ADP)-induced platelet aggregation. Error bars indicate standard deviations of the mean. LPA = late value of on-treatment platelet aggregation; LTA = light transmittance aggregometry; MPA = maximal value of on-treatment platelet aggregation.

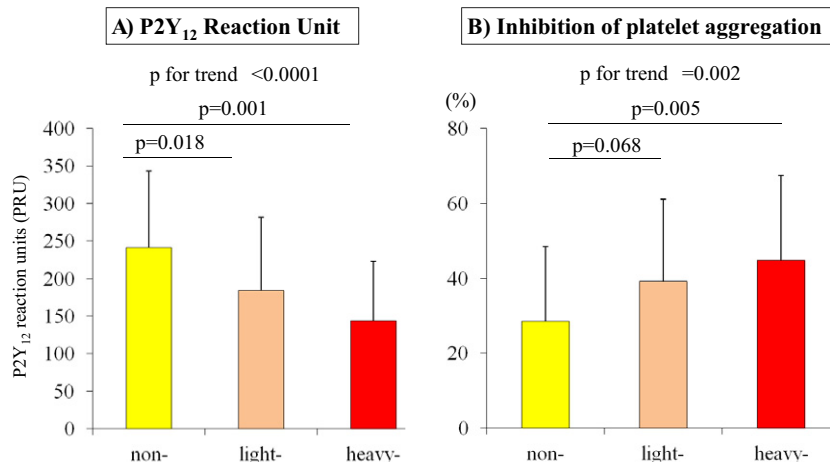


Figure 2. Platelet Reactivity Assessed by the VerifyNow P2Y₁₂ Assays According to the Degree of Smoking Defined by Cotinine Levels

Impact of the degree of smoking status to clopidogrel-induced antiplatelet effects on P2Y₁₂ reaction units (PRU) (A) and inhibition of platelet aggregation (IPA) (B). Error bars indicate SD of the mean. VerifyNow P2Y₁₂ assays are a product of Accumetrics (San Diego, California).

The prevalence of HPR in the overall study population varied according to the definition used: MPA-ADP (20 μ mol/l): 69%; MPA-ADP (5 μ mol/l): 39%; PRU: 48%; IPA: 67%; PRI: 73%. Higher serum cotinine levels were significantly associated with lower rates of HPR as defined according to all pharmacodynamic cutoff measures (Fig. 4).

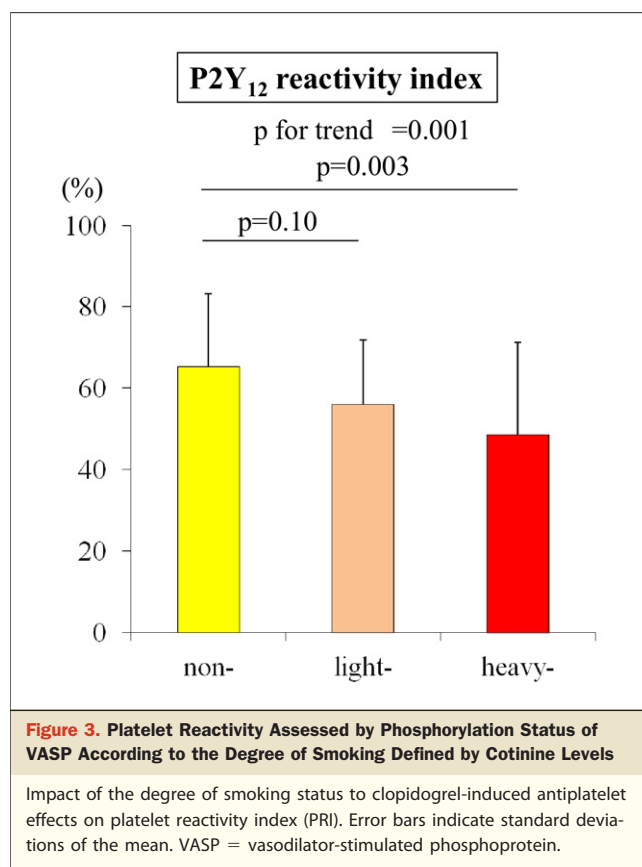
A multivariable logistic regression analysis showed that, compared with nonsmokers, light (adjusted OR: 0.24, 95% CI: 0.074 to 0.76, $p = 0.015$) and heavy smokers (adjusted OR: 0.10, 95% CI: 0.027 to 0.37, $p = 0.001$) were less likely to have HPR as assessed by LTA following 20 μ mol/l ADP stimuli. Similar results were found with 5 μ mol/l ADP stimuli (light smokers: adjusted OR: 0.47, 95% CI: 0.16 to 1.37, $p = 0.17$; heavy smokers: adjusted OR: 0.051, 95% CI: 0.006 to 0.43, $p = 0.006$), PRU values (light smokers: adjusted OR: 0.23, 95% CI: 0.063 to 0.85, $p = 0.027$; heavy smokers: adjusted OR: 0.24, 95% CI: 0.052 to 1.08, $p = 0.063$), IPA (light smokers: adjusted OR: 0.21, 95% CI: 0.062 to 0.73, $p = 0.014$; heavy smokers; adjusted OR: 0.14, 95% CI: 0.034 to 0.58, $p = 0.006$), and PRI values (light smokers: adjusted OR: 0.25, 95% CI: 0.067 to 0.94, $p = 0.039$; heavy smokers; adjusted OR: 0.24, 95% CI: 0.055 to 1.03, $p = 0.054$).

Discussion

Cigarette smoking has emerged as a factor associated with improved clopidogrel effects. This is supported by PD investigations as well as clinical outcome studies demonstrating better clopidogrel effects among smokers versus nonsmokers (6–9). However, to date, investigations have been based on self-reported smoking, which is a nonobjective way to quantify nicotine exposure. In turn, even though

these seminal investigations were able to consistently define a threshold of smoking at least one-half pack/day to significantly affect the efficacy of clopidogrel, they were not able to ascertain the presence of a dose-response effect among smokers (6–9). Cotinine is the major degradation product of nicotine metabolism and has a serum half-life of about 17 h (being detectable up to 3 days after withdrawal), and its levels correlate with the amount of nicotine exposure (i.e., severity of smoking habit) (10). To the best of our knowledge, the present investigation is the first PD study to examine and demonstrate the presence of a dose-response effect of smoking on clopidogrel effects by using a more objective measure to quantify cigarette smoking as determined by assessing serum cotinine levels. In addition to demonstrating the impact of cotinine levels on the degree of platelet reactivity, our study showed a dose-response profile on the prevalence of rates of HPR. Importantly, our findings were consistent using multiple PD parameters and confirmed in multivariate analysis, which provided support to our study hypothesis.

Multiple factors have been associated with interindividual response profiles to clopidogrel therapy (1,2). Cigarette smoking has been recently added to the factors associated with improved clopidogrel effects (6–9). The enhanced PD effects observed among smokers and the lower prevalence of HPR, defined according to cutoff values associated with recurrent atherothrombotic events, can explain why these subjects derive more benefit from clopidogrel in preventing ischemic events than nonsmokers do (7–9). The enhanced platelet inhibitory effects induced by clopidogrel among smokers can also contribute to their increased potential for bleeding complications (9,24). Reduced ischemic event rates



and increased spontaneous bleeding have also been demonstrated with novel P2Y₁₂ inhibitors characterized by more potent PD effects (25,26). Several factors can explain the “smoker’s paradox” observed among clopidogrel-treated patients. Cigarette smoking is a known inducer of CYP1A2, which is the predominant isoenzyme responsible for the first oxidative step in the conversion of clopidogrel into its active metabolite (3). Therefore, accelerating the first step of clopidogrel biotransformation would help prevent it from being shunted toward esterases mediating transformation into inactive metabolites (8). Importantly, CYP1A2 activity increases relative to the number of cigarettes smoked per day (27), which may explain the dose-response effect observed in our study.

Investigations have shown that smokers have higher P2Y₁₂ expression in platelet lysates than nonsmokers do (28). Therefore, it may be hypothesized that a high platelet surface P2Y₁₂ density may contribute to an increased risk of recurrent ischemic events among smokers, which can potentially be suppressed to a relatively greater extent by clopidogrel. Indeed, it may be argued that although several clinical studies assessing adjunctive treatment with clopidogrel in addition to aspirin in high-risk patients showed a greater relative clinical benefit in smokers than in nonsmokers (7–9), others have not (29). Differences in patient characteristics may contribute to these discrepancies as numerous

clinical characteristics have shown to affect clopidogrel metabolism and ultimately its PD effects (1,2). The present investigation was selectively conducted in patients with DM, known to have high rates of HPR (15–17,30–33). Studies have shown that this may be attributed to reduced metabolic activity of the CYP system in DM patients, which in turn generates lower levels of active metabolites than are found in non-DM patients (11). Therefore, including a population, such as patients with DM, with reduced CYP metabolic activity can increase the likelihood of identifying a dose-response effect when analyzing the impact of a CYP inducer, such as cigarette smoking. In line with this observation, recent findings have shown that the smokers’ paradox is limited only to patients with a specific *CYP1A2* genotype (34). However, the latter investigation did not discriminate the intensity of smoking in their patient population.

Despite the fact that clopidogrel effects are enhanced in smokers versus nonsmokers, cardiovascular event rates, including mortality, still remain markedly higher among smokers irrespective of type of antiplatelet treatment regimen used (35). Smoking is a major risk factor for atherothrombotic cardiovascular processes and smoking cessation is a class I recommendation for secondary prevention of ischemic events in patients with vascular disease (36). Whereas the optimal healthcare saving goal to reduce atherothrombotic risk is smoking cessation, this objective is not always achieved and many patients with established atherosclerotic disease continue smoking. Therefore, defining the optimal antiplatelet treatment strategy in these patients becomes of key importance. This is particularly relevant to those patients who do not have a clinical indication to be on dual antiplatelet therapy with aspirin and clopidogrel therapy according practice guidelines and who rely on a single antiplatelet agent, mostly aspirin, for their antithrombotic protection. Head-to-head comparisons between aspirin and clopidogrel for secondary prevention of recurrent ischemic events showed clopidogrel to be only marginally better than aspirin in the CAPRIE (Clopidogrel Versus Aspirin in Patients at Risk of Ischemic Events) trial (37). The benefit of clopidogrel was increased in higher risk subgroups, including patients with DM (38,39). Although, dedicated comparative assessments in smokers versus nonsmokers are lacking in this study, it may be hypothesized that aspirin may offer less antithrombotic protection than clopidogrel does, particularly in smokers. In fact, given the increased density of P2Y₁₂ receptors among smokers, clopidogrel may be a more effective platelet inhibitor (28). Therefore, understanding the differences in antithrombotic effects of aspirin compared with clopidogrel among smokers may help define the antiplatelet agent of choice when single therapy is indicated.

Study limitations. The present investigation is a cross-sectional observational study that evaluated the association

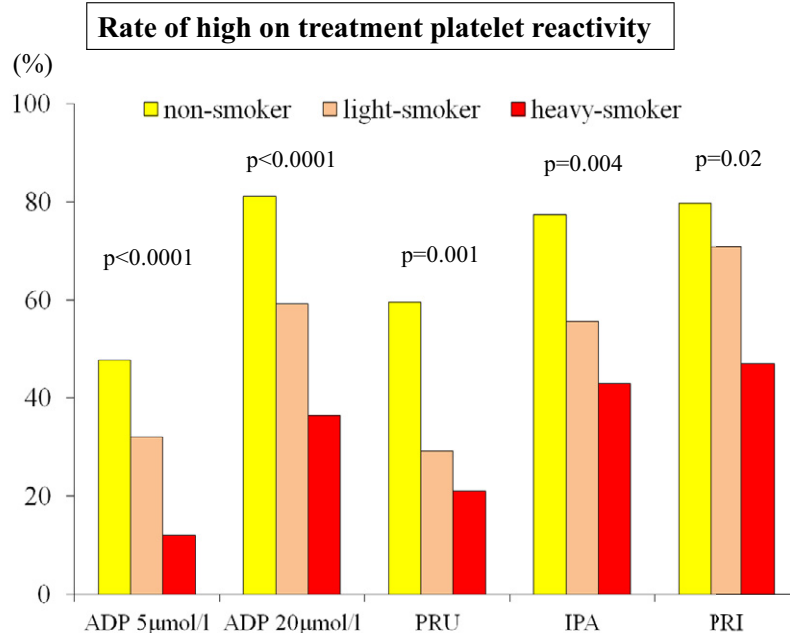


Figure 4. Rate of HPR According to the Degree of Smoking Defined by Cotinine Levels

Rates of high on-treatment platelet reactivity (HPR) assessed by different platelet function tests according to the degree of smoking defined by cotinine levels. Abbreviations as in Figures 1 to 3.

between cigarette smoking and PD effects of clopidogrel. A longitudinal study in which PD effects are measured in the same patient in the presence and absence of active cigarette smoking is needed to confirm a causative relationship between cigarette smoking and enhanced clopidogrel antiplatelet effects. The present investigation did not include pharmacokinetic assessments to determine clopidogrel active metabolite levels. In addition, the effects of smoking were not stratified according to individuals' genotype. The impact of cigarette smoking on pharmacokinetic and PD assessments, as well as if these may be affected by genotypes, is currently being investigated in a dedicated prospective trial (The Influence of Smoking Status on Prasugrel and Clopidogrel Treated Subjects Taking Aspirin and Having Stable Coronary Artery Disease; NCT01260584) that will provide further insights into this topic. A possible limitation of the present investigation is an overfitted covariate-adjusted model. However, in order to avoid spurious associations, we included in the analysis those variables that could represent potential confounders for the present analysis, as specified in the statistical analysis section.

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Key Words: clopidogrel ■ diabetes mellitus ■ platelet function ■ smoking.